Distribution of TERT promoter mutations in pediatric and adult tumors of the nervous system

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Abstract: Hot spot mutations in the promoter region of telomerase reverse transcriptase (TERT) have recently been described in several human tumor entities. These mutations result in an upregulation of the telomerase complex activity and thus constitute a relevant mechanism for immortalization of tumor cells. Knowledge of the TERT promoter status in tumors is likely to be of interest for molecular classification and as a potential target for therapy. We, therefore, performed a systematic analysis of TERT promoter mutations in 1,515 tumors of the human nervous system and its coverings including 373 pediatric and 1,142 adult patients. We detected a total of 327 mutations. TERT promoter mutations were exceedingly rare in tumors typically encountered in pediatric patients. In entities typically encountered in adult patients TERT promoter mutations were strongly associated with older age ($p < 0.0001$). Highest mutation frequencies were detected in gliosarcomas (81 %), oligodendrogliomas (78 %), oligoastrocytomas (58 %), primary glioblastomas (54 %), and solitary fibrous tumors (50 %). Related to other molecular alterations, TERT promoter mutations were strongly associated with 1p/19q loss ($p < 0.0001$), but inversely associated with loss of ATRX expression ($p < 0.0001$) and IDH1/IDH2 mutations ($p < 0.0001$). TERT promoter mutations are typically found in adult patients and occur in a highly tumor type-associated distribution.

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Distribution of *TERT* promoter mutations in pediatric and adult tumors of the nervous system

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Keywords

astrocytoma, oligodendroglioma, glioblastoma, meningioma, medulloblastoma, brain tumor, pediatric, *TERT,* promoter, mutation

Abstract

Hotspot mutations in the promoter region of *telomerase reverse transcriptase* (*TERT*) have recently been described in several human tumor entities. These mutations result in an up-regulation of the telomerase complex activity and thus constitute a relevant mechanism for immortalization of tumors cells. Knowledge of the *TERT* promoter status in tumors is likely to be of interest for molecular classification and as a potential target for therapy. We, therefore, performed a systematic analysis of *TERT* promoter mutations in 1515 tumors of the human nervous system and its coverings including 373 pediatric and 1142 adult patients. We detected a total of 327 mutations. *TERT* promoter mutations were exceedingly rare in tumors typically encountered in pediatric patients. In entities typically encountered in adult patients *TERT* promoter mutations were strongly associated with older age ($p < 0.0001$). Highest mutation frequencies were detected in gliosarcomas (81 %), oligodendrogliomas (78 %), oligoastrocytomas (58 %), primary glioblastomas (54 %) and solitary fibrous tumors (50 %). Related to other molecular alterations, *TERT* promoter mutations were strongly associated with 1p/19q loss ($p < 0.0001$), but inversely associated with loss of ATRX expression ($p < 0.0001$) and *IDH1/IDH2* mutations ($p < 0.0001$).

Together, *TERT* promoter mutations are typically found in adult patients and occur in a highly tumor type-associated distribution.

Introduction

Telomeres are composed of repetitive nucleotide sequences, which together with associated proteins constitute important structural elements at the end of chromosomes. Telomere length shortens with each cell division, and this degradation eventually leads to replicative senescence. Immortalization is a crucial bottleneck to be passed in the evolution of a cancer cell, and the ability to maintain telomere length is therefore a typical feature of neoplasia. Telomeres are extended by the protein complex telomerase, in which the enzyme telomerase reverse transcriptase (*TERT*) plays a pivotal role [33]. Previous studies have shown a strong expression of TERT in the vast majority of human cancers [29], although the underlying mechanisms of TERT up-regulation were largely unknown. Recently, several alterations within the *TERT* gene have been detected in human tumors. Single nucleotide polymorphisms in the *TERT* gene have been associated with telomere length and have been associated with risk for breast and ovarian cancer [3]. Also the methylation status of distinct CpG sites in the *TERT* promoter has been associated with TERT expression, tumor progression and poor prognosis in pediatric nervous system tumors [5]. The most frequent alteration in the *TERT* gene, somatic promoter mutations, have been described in melanoma [9,12,13], primary nervous system tumors [2,14,17,23,30], thyroid [15,16], hepatocellular carcinomas [21] and bladder [17] as well as many other tumor types [14,30]. Two essentially mutually exclusive hot spot mutations in the promoter region have been reported: a cytosine-to-thymine transition at chromosome 5 base position 1,295,228 (C228T) or less frequently at base position 1,295,250 (C250T). Both changes create a new binding site for E-twenty-six (ETS) transcription factors, resulting in an up to 5-fold increased induction of the *TERT* gene [13].

Another mechanism known relevant for maintaining telomere length is alternate lengthening of telomeres (ALT) [4] which is a homologous recombination dependent process typically associated with mutations in the chromatin remodeling genes *α-thalassemia/mental-retardation-syndrome-X-linked* (*ATRX*) and *death-associated protein 6* (*DAXX*) [11]. ALT has been demonstrated in gliomas and other tumors [7,8]. However, *TERT* promoter mutations appear to be the single most frequently occurring mechanism to affect telomere length.

So far, the *TERT* promoter status has been published for 1507 nervous system tumors [2,14,17,23,30]. The vast majority of these tumors were from adult patients and 843/1507 tumors have been diagnosed as glioblastoma (supplementary table 1). In order to provide a comprehensive overview of the frequency of *TERT* promoter mutations we analyzed 1515 nervous system tumors covering a wide range of diagnoses from 373 pediatric and 1142 adult patients [19].

Material and Methods

Tissue samples

Tumor tissues for DNA extraction were obtained from the archives of the Institutes and Departments of Neuropathology at the University Hospitals Münster, Hannover, Frankfurt, Tübingen, Magdeburg and Heidelberg (Germany). Research use of tissues and anonymisation of data was in accordance with local ethical approvals. Tumors from patients younger than 18 years of age were termed pediatric and tumors from patients aged 18 or older were termed adult. Diagnosis, age and *TERT* promoter mutation status for each patient are given in supplementary table 2. Further molecular data is summarized for astrocytic tumors, oligododendroglial tumors and medulloblastomas in supplementary tables 3-5.

DNA-based methods

Amplicons of 163 bp spanning the hot-spot mutations at positions 1,295,228 and 1,295,250 on chromosome 5 were amplified using 20 ng each of the forward primer 5`CAGCGCTGCCTGAAACTC and the reverse primer 5`GTCCTGCCCCTTCACCTT. Primer design was based on accession number NC_000005.9. 80 ng of DNA and AccuPrime™ GC-Rich DNA Polymerase kit (Life Technologies, Darmstadt, Germany) were employed. PCR was performed in a total volume of 25 µl, and included initial denaturation at 95°C for 180 s, followed by 35 cycles with denaturation at 95°C for 30 s, annealing at 62°C for 25 s and extension at 72°C for 30 s. The amplification product was purified and cleaned up by agarose gel electrophoresis. Subsequently the 163 bp products were gel-extracted with the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany) according to the manufacturers' protocol. Two µl of the purified amplification product was submitted to bidirectional sequencing using the BigDye Terminator v3.1 Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequences were determined using an ABI 3500 Genetic Analyzer (Applied Biosystems) and the Sequence Pilot version 4.0.1 (JSI-Medisys, Kippenheim, Germany) software.

Determination of hotspot mutations in *BRAF* and *IDH1/2,* and testing for 1p/19q LOH was carried out as previously described [10,25,26].

ATRX immunohistochemistry

ATRX staining was performed as previously described [32]. In brief, ATRX polyclonal rabbit antibody (dilution 1:400, product code HPA001906, Sigma-Aldrich, St. Louis, MO, USA) was used. Staining was performed using an automated immunostainer (Benchmark Ultra, Ventana, Tucson, AZ, USA) and standard protocols including pretreatment using Cell Conditioning 1 buffer (Ventana) for 52 min and standard Ventana signal amplification. ATRX data have been reported in part previously [32].

Statistics

Fisher's exact test was used to examine associations between nominal variables. Student's t test was used to examine the association between nominal variables and age.

Results and Discussion

This study presents a comprehensive overview of *TERT* promoter mutations in tumors of the nervous system and its coverings. A total of 1515 tumors were tested and 327 *TERT* promoter mutations were found. The C228T mutation was detected 257 times making it more frequent than the C250T mutation seen in 68 cases. Two further mutation variants were sporadically observed: a pediatric glioblastoma with C228A mutation and a pleomorphic xanthoastrocytoma with C249T mutation. A summary of the pediatric and adult tumors and corresponding mutation rates for the *TERT* promoter are given in table 1. Although *TERT* promoter mutations over-all were very frequent, we also detected significant differences between distinct tumor entities:

Astrocytic tumors

TERT promoter mutations were not detected in 111 pilocytic astrocytomas WHO grade I. Likewise, the rare variants of subependymal giant cell astrocytoma WHO grade I and pilomyxoid astrocytoma WHO grade II did not show this alteration. Pleomorphic xanthoastrocytomas WHO grade II (PXA II) had a low frequency of mutations with a single case out of 25 tested tumors bearing a unique C249T mutation. Pleomorphic xanthoastrocytomas with anaplastic features (PXA waf) exhibited *TERT* promoter mutations in 3/13 instances. All three patients were of older age. *TERT* promoter hotspot mutations create a new binding site for ETS transcription factors, which are partly regulated by the mitogen-activated protein kinase (MAP kinase) signaling pathway, in which BRAF acts as an upstream signaling kinase [31]. Furthermore, *BRAF* V600E is a hallmark mutation in PXAs [26] and leads to aberrant MAPK signaling [6]. Coincidental occurrence of *BRAF* V600E and *TERT* promoter mutations has been described in thyroid cancers [15,16]. However, in PXAs there was no association between *TERT* promoter and *BRAF* V600E mutation ($p = 0.6$; suppl. fig. 1a).

Diffuse astrocytomas WHO grade II exhibited *TERT* promoter mutations in 8/25 cases and showed a strong inverse association with the presence of *IDH1*/*IDH2* mutations ($p < 0.05$; suppl. fig. 1b) [14]. Similar observations were made for anaplastic astrocytoma WHO grade III with *TERT* promoter mutations in 30/118 cases, also inversely associated with *IDH1*/*IDH2* ($p < 0.0001$; suppl. fig. 1c). ATRX alterations have been shown to closely overlap with *IDH1*/*IDH2* and *TP53* mutations in astrocytoma [18,32]. Likewise, we observed an inverse association between loss of ATRX expression and *TERT* promoter mutations ($p < 0.05$; suppl. fig. 1d). Primary glioblastoma in adults exhibited a high incidence of *TERT* promoter mutations (79/147, 54 %) while only 1/20 secondary glioblastomas had a mutation. For primary glioblastoma this matches previous observations and supports the inverse association of *TERT* promoter and *IDH* mutations. Our mutation frequency of 5 % in secondary glioblastomas is lower than the 28 % previously observed [23] although, the number of secondary glioblastomas in our series is rather small. The observation of *TERT* mutations in 33 % of diffuse astrocytomas and 28 % of anaplastic astrocytomas would also suggest a higher frequency of this alteration in secondary glioblastomas than identified in our series. The rare and controversially discussed glioblastoma with oligodendroglial differentiation exhibited *TERT* promoter mutation in 4/6 cases. Because *TERT* promoter mutations are frequent in both, primary glioblastoma and anaplastic oligodendroglioma, this parameter most likely will not be helpful to contribute to the debate on the

origins of glioblastoma with oligodendroglial differentiation. Giant cell glioblastomas were mutated in 6/17 cases. This variant has been shown to be strongly associated with *TP53* mutations [20,24]. Gliosarcomas carried *TERT* promoter mutations in 21/26 instances, the highest mutation prevalence in our series. Among gliomatoses 7/10 tumors harbored the mutation.
In opposition to adult patients, *TERT* promoter hotspot mutations were not present in pediatric primary glioblastomas except one case with a unique C228A mutation. Constitutional DNA from this patient was not available. However, single somatic C228A mutations have already been described in one ovarian carinoma and in two urothelial carcinomas [14]. In contrast to the C228T and C250T changes, the C228A alteration does not create the new binding site for ETS transcription factors. However, an effect of this alteration on binding of other transcription factors cannot be excluded. Our pediatric GBM with C228A mutation was wild type for *H3F3A* frequently been mutated in pediatric glioblastomas and typically associated with *ATRX* mutation and ALT characteristics [27]. *ATRX* mutations frequently occur in pediatric GBMs [27] and, in adult glioma, are mutually exclusive with *TERT* promoter mutation [2]. We detected ATRX mutation/loss of expression in 3/11 pediatric glioblastomas (fig. 1). Current data regarding the association of ALT status and ATRX loss in adult GBMs is controversial. While one series detected the presence of ALT in approximately 25 % [1] two other series detected ALT in less than 14 % [22] and 7 % [18] of adult GBM. Also, one series did not find a difference in frequency of ALT between adult and pediatric GBM [1] while another series found this alteration 3 times more frequent in pediatric glioblastoma [22]. While two studies found a tight correlation between ALT phenotype and loss of ATRX expression [1,22], another did not [18]. More comprehensive and histopathologically clearly defined series of tumors need to be examined in order to resolve these discrepancies.

Oligodendroglial tumors

TERT promoter mutations were found in 29/37 oligodendrogliomas WHO grade II and in 29/38 anaplastic oligodendrogliomas WHO grade III. Oligoastrocytomas WHO grade II presented with *TERT* promoter mutations in 11/19 instances, while the incidence in anaplastic oligoastrocytomas WHO grade III was 45/86. Therefore, oligoastrocytomas had a lower prevalence of *TERT* promoter mutations than pure oligodendrogliomas. However, there was no increase of mutation frequency with increasing malignancy grade in oligodendroglial tumors. *TERT* mutations were frequent in oligodendroglial tumor entities, regardless of the WHO grade. Previous studies have highlighted the strong association of 1p/19q loss and *TERT* promoter mutations [2,14]. This is consistent with our series, in which oligodendroglial tumors with a combined 1p/19q loss were significantly more frequently *TERT* promoter mutated (49/56; 88 %) compared with oligodendroglial tumors with 1p/19q retention (8/22; 36 %) ($p < 0.0001$; suppl. fig. 1e). Loss of ATRX expression was observed in 13/55 oligodendroglial tumors and inversely associated with *TERT* promoter mutations. Vice versa 28/42 cases with retained ATRX expression were *TERT* promoter mutated ($p < 0.0001$; suppl. fig. 1f). 10/14 ATRX expressing cases with *TERT* promoter wild type status were oligoastrocytomas.

Ependymal tumors

TERT promoter mutations were found in 0/12 subependymomas WHO grade I and 0/14 myxopapillary ependymomas WHO grade I. 5/73 ependymomas WHO grade II and 2/46 anaplastic ependymomas WHO grade III had the *TERT* promoter mutation. All *TERT* mutations in ependymomas occurred in adult patients with these seven patients being aged between 55 and 67 years. Thus, *TERT* promoter mutations were absent in all pediatric ependymoma and in two subgroups of ependymoma graded WHO I i.e. the subependymomas and the myxopapillary ependymomas. Adult patients with WHO grade II and WHO grade III anaplastic ependymoma younger than 50 years did not carry *TERT* mutations while more than 25 % of the ependymoma patients 50 years or older had a *TERT* promoter mutation.

Embryonal tumors

In concordance with previous data [14] we detected *TERT* promoter mutations in 17/91 medulloblastomas. 15 of the 17 patients with *TERT* mutations in medulloblastoma were of adult age and 15 of the mutated tumors were allotted to the SHH group of medulloblastoma. Thus, while rare in pediatric medulloblastomas (3 %), *TERT* mutations were very frequent in adult medulloblastomas (65 %) and particular in adult SHH subgroup tumors (13/13, 100 %). In this patient set, we observed a strong prevalence of the C228T mutation. *TERT* promoter mutations were absent or seen only at very low frequency in all other embryonal tumor entities.

Meningeal tumors

Previous investigations have found no *TERT* promoter mutations in meningiomas [14]. Accordingly we detected no *TERT* promoter mutations in 91 meningiomas WHO grade I of different subtypes. However, in atypical (WHO grade II) and anaplastic/rhabdoid meningiomas (WHO grade III) we detected mutations in 2/49 and 6/37 instances, respectively. Thus, the frequency of *TERT* promoter mutations increased with higher malignancy. Hemangiopericytomas (HPC) and solitary fibrous tumors (SFT) have recently been shown to share the recurrent NAB2-STAT6 fusion that leads to a strong nuclear translocation of STAT6 [28]. This molecular hallmark alteration now allows for a sharp separation of HPCs/SFTs from atypical and anaplastic meningiomas. Since SFT/HPC both shared this gene fusion, we expected no pronounced difference in *TERT* promoter mutation frequency between the two entities. In a previously analyzed smaller series of SFT/HPC 2 of 10 cases showed *TERT* promoter mutations [14]. Interestingly, we detected mutations in only 3/27 HPCs but in 8/16 SFTs. This difference in *TERT* promoter mutation frequency between HPC and SFT is unexplained. No *TERT* promoter mutation was observed in 13 hemangioblastomas WHO grade I.

Other tumors of the nervous system

The other tumor groups as defined by WHO, including choroid plexus tumors, neuronal and mixed neuronal-glial tumors, tumors of the pineal region, tumors of the cranial and paraspinal nerves, tumors of the hematopoietic system with primary lymphomas and histiocytic tumors, germ cell tumors and tumor of the sellar region exhibited *TERT* promoter mutations in only a few single instances (table 1). For several of the rare entities, however, only low numbers of tumors were available for analysis.

TERT mutations in adult and pediatric patients with nervous system tumors

A clear accumulation of *TERT* promoter mutations was seen in adult astrocytomas, glioblastomas and oligodendroglial tumors. Also the less frequent *TERT* promoter mutations in ependymoma exclusively occurred in adult patients of older age. A further group of special interest is adult patients with medulloblastoma, who in contrast to their pediatric counterparts exhibited a high incidence of *TERT* mutations. In addition, there also seems to be some association of *TERT* mutations with higher grades of malignancy in meningioma.

In contrast to adults, pediatric patients very rarely exhibited *TERT* promoter mutations. Overall only 7 *TERT* promoter mutations were observed in 373 pediatric tumors. This is of special interest in the case of tumors which occur across ages, e.g. medulloblastoma, glioblastoma and ependymoma, where *TERT* mutations were quite frequently observed in adult patients but not in children. The most frequent pediatric nervous system tumor, pilocytic astrocytoma WHO grade I also did not exhibit *TERT* promoter mutations.

We analyzed the association of *TERT* promoter status with age for those entities with high *TERT* promoter mutation frequencies and with high numbers of patients included. We pooled astrocytomas WHO grade II and III, oligoastrocytomas WHO grade II and III, and oliogdendrogliomas WHO grade II and III because *TERT* promoter mutation frequencies were independent of grade within these entities. *TERT* promoter mutations were strongly confined to older age in glioblastomas ($p < 0.001$), astrocytomas ($p < 0.001$), oligoastrocytomas ($p < 0.05$), oligodendrogliomas ($p < 0.05$) and medulloblastomas ($p < 0.001$) (fig. 2 a-f).

Our findings support that immortalization of tumor cells in pediatric nervous system tumor patients generally is not achieved by *TERT* promoter mutation mediated activation of telomerase. However, other mechanisms of telomerase activation might very well be of importance in this age group as demonstrated by a recent publication associating the methylation status of *TERT* with prognosis in pediatric nervous system tumors [5], and by the finding of ALT in a high proportion of pediatric glioblastomas [27].

Conclusion

TERT promoter mutations predominantly occur in glial tumors of adult patients. They are most frequent in astrocytic and oligodendroglial tumors of WHO grade II and higher. In contrast, pediatric nervous system tumor patients rarely have *TERT* promoter mutations. In those instances where *TERT* mutations were found in typical pediatric tumors, the patients were of older age.

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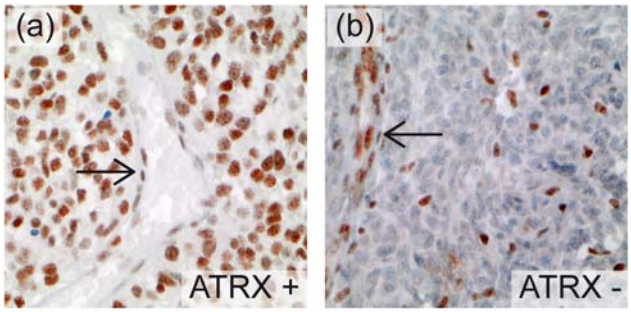
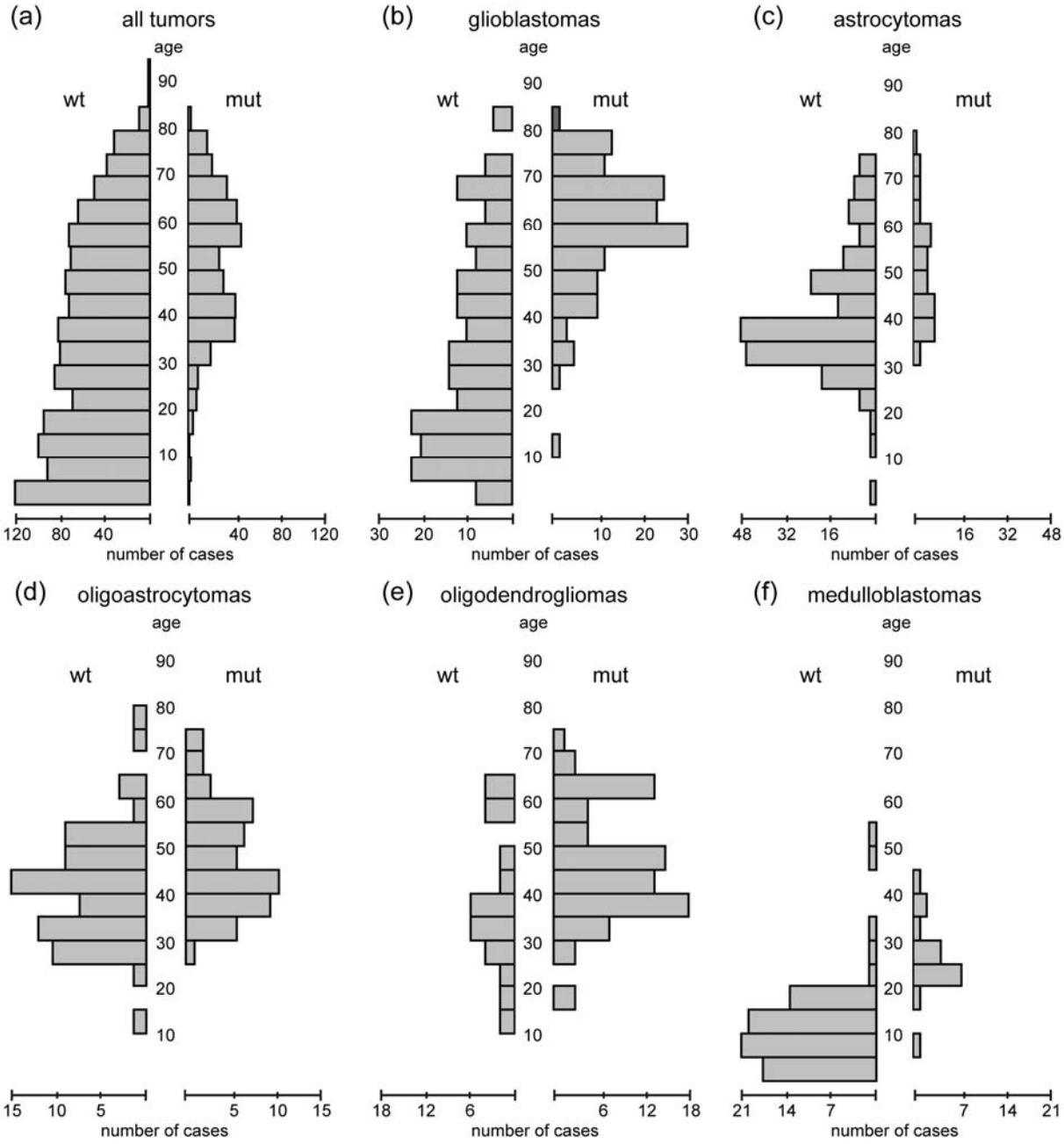
Figure 1



ATRX immunohistochemistry in a pediatric glioblastoma WHO grade IV (ID 63556) with nuclear expression of ATRX (a) and a pediatric glioblastoma WHO grade IV (ID 63566) with loss of nuclear ATRX expression but ATRX presence in reactive cells and endothelial cells (b). Vessels (arrow) serve as internal positive control. Magnification: 400-fold

Figure 2



TERT promoter mutation status (wt/mut) in relation to age (years). *TERT* promoter mutation was significantly associated with younger patient age based on (a) all tumors ($p < 0.0001$), (b) glioblastomas ($p < 0.0001$), (c) astrocytomas WHO grade II and III ($p < 0.0001$), (d) oligoastrocytomas WHO grade II and III ($p < 0.05$), (e) oligodendrogliomas WHO grade II and III ($p < 0.05$), and (f) medulloblastomas ($p < 0.0001$).

Table 1

C228T and C250T *TERT* promoter mutations in tumors of the nervous system

Tumor group	Diagnosis (WHO grade)	all **N** ; mut/wt % (C228/C250)	pediatric (<18 years) **N** ; mut/wt % (C228/C250)	adult (>=18 years) **N** ; mut/wt % (C228/C250)
Astrocytic tumors	Pilocytic Astrocytoma (I)	**111**; 0/111	**90**	**21**
	Pilomyxoid Astrocytoma (II)	**3**;0/3	**1**	**2**
	Subependymal Giant Cell Astrocytoma (I)	**10**;0/10	**5**	**5**
	Pleomorphic Xanthoastrocytoma (II)	**25**; 1*/24 4%	**10**; 0/10	**15**; 1*/14
	Pleomorphic Xanthoastrocytoma with anaplastic features	**13**; 3/10 23% (2/1)	**2**	**11**; 3/8 (2/1)
	Diffuse Astrocytoma (II)	**25**; 8/17 32% (4/4)	**1**; 0/1	**24**; 8/16 33% (4/4)
	Anaplastic Astrocytoma (III)	**118**; 30/88 25% (24/6)	**1**; 0/1	**117**; 30/87 26% (24/6)
	Glioblastoma primary (IV)	**147**; 78/68+1** 54% (66/12)	**32**; 1**/31 3%	**115**; 78/37 68% (66/12)
	Glioblastoma secondary (IV)	**20**; 1/19 5% (0/1)	**-**	**20**; 1/19 5% (0/1)
	Giant Cell Glioblastoma (IV)	**17**; 6/11 35% (4/2)	**-**	**17**; 6/11 35% (4/2)
	Glioblastoma with oligodendroglial differentiation (IV)	**6**; 4/2 (3/1)	**-**	**6**; 4/2 (3/1)
	Gliosarcoma (IV)	**26**; 21/5 81% (17/4)	**-**	**26**; 21/5 81% (17/4)
	Gliomatosis	**10**; 7/3 70% (7/0)	**1**; 1/0 (1/0)	**9**; 6/3 66% (6/0)
Oligodendroglial tumors	Oligodendroglioma (II)	**37**; 29/8 78% (21/8)	**2**; 1/1 (0/1)	**35**; 28/7 80% (21/7)
	Anaplastic Oligodendroglioma (III)	**38**; 29/9 76% (20/9)	**-**	**38**; 29/9 76% (20/9)
	Oligoastrocytoma (II)	**19**; 11/8 58% (9/2)	**1**; 0/1	**18**; 11/7 61% (9/2)
	Anaplastic Oligoastrocytoma (III)	**86**; 45/41 52% (37/8)	**-**	**86**; 45/41 52% (37/8)

Ependymal tumors	Subependymoma (I)	**12**; 0/12	**-**	**12**
	Myxopapillary Ependymoma (I)	**14**; 0/14	**1**	**13**
	Ependymoma (II)	**73**; 5/68 7% (5/0)	**25**; 0/25	**48**; 5/43 10% (5/0)
	Anaplastic Ependymoma (III)	**48**; 2/46 4% (1/1)	**28**; 0/28	**20**; 2/18 10% (1/1)
Choroid plexus tumors	Plexus Papilloma	**13**; 0/13	**3**	**10**
	Plexus Carcinoma	**6**; 1/5 (1/0)	**3**; 0/3	**3**; 1/2 (1/0)
Other neuroepithelial tumors	Angiocentric Glioma (I)	**9**; 0/9	**5**	**4**
Neuronal and mixed neuronal-glial tumors	Desmoplastic Infantile Astrocytoma (I)	**2**; 0/2	**2**	**-**
	Desmoplastic Infantile Ganglioglioma (I)	**6**; 1/5 (1/0)	**6**; 1/5 (1/0)	**-**
	Dysembryoplastic Neuroepithelial Tumor (I)	**12**; 0/12	**5**	**7**
	Ganglioglioma (I)	**40**; 1/39 2% (1/0)	**17**; 0/17	**23**; 1/22 (1/0)
	Anaplastic Ganglioglioma (III)	**3**; 0/3	**-**	**3**
	Gangliocytoma (I)	**2**; 0/2	**1**	**1**
	Neurocytoma (II)	**28**; 1/27 4% (1/0)	**2**; 0/2	**26**; 1/25 4% (1/0)
	Papillary Glioneuronal Tumor (I)	**1**; 0/1	**1**	**-**
	Rosette-forming Glioneuronal Tumor of the fourth Ventricle (I)	**6**; 0/6	**1**	**5**
	Paraganglioma (I)	**12**; 1/11 8% (1/0)	**-**	**12**; 1/11 8% (1/0)
Tumors of the pineal region	Pineocytoma (I)	**2**; 0/2		**2**
	Pineal Parenchymal Tumor of Intermediate Differentiation (II)	**9**; 0/9	**-**	**9**
	Pineoblastoma (IV)	**5**; 0/5	**1**	**4**
	Papillary Tumor of the Pineal Region (II)	**2**; 0/2	**-**	**2**

Embryonal tumors	Medulloblastoma (IV)	**91**; 17/75 18% (16/1)	**68**; 2/66 3% (1/1)	**23**; 15/8 65% (15/0)
	Ependymoblastoma/ETANTR/ETMR (IV)	**13**; 0/13	**13**	**-**
	Atypical Teratoid / Rhabdoid Tumor (IV)	**19**; 1/18 6% (1/0)	**19**; 1/18 6% (1/0)	**-**
	Esthesioneuroblastoma	**12**; 0/12	**-**	**12**
Tumors of the cranial and paraspinal nerves	Schwannoma (I)	**18**; 0/18	**-**	**18**
	Neurofibroma (I)	**17**; 0/17	**1**	**16**
	Malignant Peripheral Nerve Sheath Tumor (IV)	**12**; 2/10 17% (1/1)	**-**	**12**; 2/10 17% (1/1)
Meningeal tumors	Meningioma meningothelial (I)	**19**; 0/19	**-**	**19**
	Meningioma transitional (I)	**25**; 0/25	**-**	**25**
	Meningioma fibroblastic (I)	**11**; 0/11	**-**	**11**
	Meningioma psammomatous (I)	**11**; 0/11	**-**	**11**
	Meningioma secretory (I)	**17**; 0/17	**-**	**17**
	Meningioma angiomatous (I)	**8**; 0/8	**-**	**8**
	Meningioma atypical (II)	**42**; 2/40 5% (2/0)	**-**	**42**; 2/40 5% (2/0)
	Meningioma chordoid (II)	**5**; 0/5	**-**	**5**
	Meningioma clear cell (II)	**2**; 0/2	**1**	**1**
	Meningioma anaplastic (III)	**32**; 4/28 13% (3/1)	**-**	**32**; 4/28 13% (3/1)
	Meningioma rhabdoid (III)	**5**; 2/3 (1/1)	**1**	**4**; 2/2 (1/1)
	Hemangiopericytoma	**27**; 3/24 11% (2/1)	**-**	**27**; 3/24 11% (2/1)
	Solitary Fibrous Tumor	**16**; 8/8 50% (6/2)	**-**	**16**; 8/8 50% (6/2)
	Hemangioblastoma (I)	**13**; 0/13	**1**	**12**
Tumors of the hematopoietic system	Lymphomas	**12**; 0/12	**2**	**10**
	Histiocytic Tumors	**18**; 0/18	**6**	**12**

Germ cell tumors	Germinoma	**7**; 0/7	**5**	**2**
	Teratoma	**4**; 0/4	**3**	**1**
	Yolk Sac Tumor	**4**; 0/4	**1**	**3**
	Choriocarcinoma	**4**; 1/3 (0/1)	**-**	**4**; 1/3

Tumors of the sellar region	Craniopharyngioma	**18**; 0/18	**4**	**14**
	Granular Cell Tumor	**5**; 1/4 (0/1)	**1**; 0/1	**4**; 1/3 (0/1)
	Pituicytoma	**1**; 0/1	**-**	**1**
	Pituitary Adenoma	**11**; 0/11	**-**	**11**

* - pleomorphic xanthoastrocytoma with a C249T mutation; ** - pediatric glioblastoma with a C228A mutation

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